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Protective role of frizzled-related protein B on matrix metalloproteinase induction in mouse chondrocytes

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Abstract

Introduction

Our objective was to investigate whether a lack of frizzled-related protein B (FrzB), an extracellular antagonist of the Wnt signaling pathways, could enhance cartilage degradation by facilitating the expression, release and activation of matrix metalloproteinases (MMPs) by chondrocytes in response to tissue-damaging stimuli.

Methods

Cartilage explants from $FrzB^{-/-}$ and wild-type mice were challenged by excessive dynamic compression (0.5 Hz and 1 MPa for 6 hours). Load-induced glycosaminoglycan (GAG) release and MMP enzymatic activity were assessed. Interleukin-1β (IL-1β) (10, 100 and 1000 pg/mL for 24 hours) was used to stimulate primary cultures of articular chondrocytes from $FrzB^{-/-}$ and wild-type mice. The expression and release of MMP-3 and −13 were determined by RT-PCR, western blot and ELISA. The accumulation of β-catenin was assessed by RT-PCR and western blot.

Results

Cartilage degradation, as revealed by a significant increase in GAG release (2.8-fold, $P = 0.014$) and MMP activity (4.5-fold, $P = 0.014$) by explants, was induced by an excessive load. Load-induced MMP activity appeared to be enhanced in $FrzB^{-/-}$ cartilage explants compared to wild-type ($P = 0.17$). IL-1β dose-dependently induced *Mmp-13* and *−3* gene expression and protein release by cultured chondrocytes. IL-1β-mediated increase in MMP-13 and −3 was slightly enhanced in $FrzB^{-/-}$ chondrocytes compared to wild-type ($P = 0.05$ and $P = 0.10$ at gene level, $P = 0.17$ and $P = 0.10$ at protein level, respectively). Analysis of *Ctnn1b* and *Lef1* gene expression and β-catenin accumulation at protein level suggests that the enhanced catabolic response of $FrzB^{-/-}$ chondrocytes to IL-1β and load may be associated with an over-stimulation of the canonical Wnt/β-catenin pathway.

Conclusions

Our results suggest that FrzB may have a protective role on cartilage degradation and MMP induction in mouse chondrocytes by attenuating deleterious effects of the activation of the canonical Wnt/β-catenin pathway.

Introduction

Osteoarthritis (OA) is a common multifactorial joint disease, with ageing and excessive loading as important risk factors. Although the pathophysiology of OA involves cartilage, bone and the synovial tissue, the main feature of OA remains the progressive degradation of articular cartilage. Progressive joint destruction in OA has been associated with over-activation of Wnt signaling in numerous studies [1-6]. The Wnt signaling pathway is a potent regulator of bone and cartilage homeostasis and also has a role in human joint diseases [7,8]. The canonical Wnt pathway is initiated by binding of Wnt ligands to frizzled receptors and co-receptors “low-density lipoprotein receptors” (LRP-5/6), which leads to intracellular β-

catenin stabilization and accumulation, nuclear translocation, interaction with transcription factors, T-cell factor and lymphoid enhancer binding factor (TCF/LEF), and finally activation of target genes. The non-canonical Wnt pathways involve specific ligands and are independent of β-catenin and LRPs. Wnt ligands such as Wnt-7b, Wnt-16 and Wnt target gene "Wnt-1 inducible signaling pathway protein 1" (Wisp-1) were found up-regulated in OA cartilage [1-3]. In addition, β-catenin, the co-receptor LRP-5 and the transcription factor LEF-1 were found over-expressed [4-6]. FrzB (Frizzled-related protein) is an extracellular antagonist of the Wnt signaling pathway, also called secreted Frizzled-related protein 3 (sFRP-3). As FrzB can bind Wnts in the extracellular space and prevent ligand-receptor interaction, it can be considered an antagonist of both canonical and non-canonical signaling. Two single nucleotide polymorphisms in *FRZB*, which are loss-of-function mutations, were associated with an increased risk of OA [9-13]. However, this association was challenged by recent studies [14-17], so that the potential role of FrzB in OA is still controversial.

Studies in mouse models of OA corroborated the association between cartilage degradation and an over-activation of Wnt signaling. In particular, Wisp-1 was found up-regulated in 2 models of OA; moreover, local over-expression of Wisp-1 enhanced cartilage damage [3]. Transgenic mice that produced activated-β-catenin in adult chondrocytes developed an OA-like phenotype upon ageing [4]. $FrzB^{-/-}$ knockout mice did not develop spontaneous OA, however the deletion of *FrzB* increased cartilage loss in 3 different models of arthritis [18]. Thus, FrzB may have a protective role on OA progression. How FrzB can influence OA process remains largely unclear, but various hypotheses have been suggested [4,18-22]. The canonical Wnt pathway is crucial for proper chondrocyte differentiation in early developmental processes to control chondrogenesis and later, to regulate hypertrophic maturation. Abnormal Wnt signaling in the absence of FrzB could cause aberrant skeletal morphogenesis and variations in human hip shape have been associated with the above-mentioned FrzB polymorphisms [19]. This could contribute to the development of OA by increasing the biomechanical burden on the articular cartilage [19,23]. In addition, OA is also characterized by hypertrophy-like changes in chondrocytes, which could be enhanced by an over-activation of Wnt signaling in absence of FrzB [24].

In cultured chondrocytes, Wnt-3a, a commonly used Wnt ligand that triggers β-catenin signaling, increased the expression of MMP-3 and −13, and MMP-2 and −9 enzymatic activities [25-27]. In transgenic mice, activated-β-catenin increases the expression of MMP-2, −3, −7, −9 and −13 [4,28]. Similarly, down-regulation of LRP-5 decreased the expression of MMP-7, −9, −13 and −14 [5,6]. The transcription complex formed by activated-β-catenin and Lef-1 has been shown to strongly bind MMP-9, −13 and −14 promoters, especially [5,29].

We focused on the hypothesis that the absence of FrzB could favor OA-like catabolic processes in cartilage by increasing the activation of the Wnt signaling pathway. We therefore studied cartilage degradation in FrzB KO mice, after biomechanical loading or cytokine treatment.

Methods

Animals

All experiments were made on explants or primary chondrocytes extracted from 3 to 6-day-old newborn litters from $FrzB^{-/-}$ or wild-type mice [18]. All procedures were in accordance

with the European Directive N886/609 and were performed according to the protocols approved by French and European ethics committees for animal use and care (Comité Régional d'Ethique en Expérimentation Animale N°3 de la région Ile de France).

Compression of costal cartilage explants

The procedure for compressive loading of mouse costal cartilage explants was as described [30]. Briefly, explants were harvested from rib cages of 4- to 6-day-old newborn mice. Samples were cleaned, divided into segments, pooled and weighed for further normalization; each sample consisted of around 30–40 mg of costal cartilage. The explants were allowed to rest for about 20 h in 3 mL of serum-free medium (DMEM medium supplemented with 0.1% bovine serum albumin and 30 mM Hepes). They were washed before they underwent 6-h dynamic compression in 1.5 mL of the same fresh medium (sinusoidal wave-form 0–1 MPa at 0.5 Hz) by the Biopress system (Flexercell International, Dunn Labortechnick GmbH, Asbach, Germany). Control explants were kept in unloaded conditions. After the application of the mechanical regimen, supernatants and cartilage explants were collected and stored immediately at −80°C. Our results are expressed as fold-induction versus the uncompressed explants controls.

Primary culture of mouse articular chondrocytes

Primary chondrocytes were isolated from articular cartilage of 4- to 6-day-old newborn mice as described [31]. After 1 week of expansion, the cells were placed in serum-free conditions for 24 h (0.1% bovine serum albumin, Sigma Aldrich, Saint-Quentin Fallavier, France), then treated for 24 h in serum-free medium supplemented with 0, 10, 100 or 1000 pg/mL IL-1β (PeproTech, Tebu-Bio, Le Peray-en-Yvelines, France). Our results are expressed as fold-induction versus the non-treated controls.

RNA extraction, RT and real-time PCR

RNA was extracted from chondrocytes cultured with and without IL-1β by use of the RNeasy minikit (Qiagen, Courtaboeuf, France); for the RNA extraction from compressed and uncompressed cartilage explants, Proteinase K (Qiagen) was first added to remove proteins as suggested for tissue samples by the manufacturer (Proteinase K, Qiagen). Reverse transcription was performed on 1 μg RNA for monolayer-cultured cells and 100–200 ng RNA for explants by use of the Omniscript kit (Qiagen). Relative quantification of genes involved use of the LC480 LightCycler Real Time PCR system (Roche Applied Science, Meylan, France) and Go Taq QPCR master mix (Promega, Charbonnières les Bains, France). mRNA levels of MMP-3 and −13, β-catenin (*Ctnnb1*) and Lef1 were normalized to that of hypoxanthine-guanine phosphoribosyltransferase (*Hprt*), used as internal standard.

Mmp-3 s-TGAAAATGAAGGGTCTTCCGG; as-GCAGAAGCTCCATACCAGCA,

Mmp-13 s-GATGGCACTGCTGACATCAT; as-TGTAGCCTTTGGAACTGCTT,

Ctnnb1 s-GCAGCAGCAGTTTGTGGA; as-TGTGGAGAGCTCCAGTACACC,

Lef1 s-TCCTGAAATCCCCACCTTCT; as-TGGGATAAACAGGCTGACCT,

Hprt s-AGGACCTCTCGAAGTGT; as-ATTCAAATCCCTGAAGTACTCAT.

MMP-3 and −13 protein measurement

The amount of MMP-3 and −13 released by chondrocytes in response to IL-1β was assessed in the culture supernatants. For total mouse MMP-3 secretion we used a commercially available ELISA kit (R&D systems, Lille, France). For total mouse MMP-13 secretion we performed western blot analysis as described [32] with rabbit polyclonal antibody for MMP-13 (H-230) (Santa Cruz Biotechnology, Tebu-Bio, Le Peray-en-Yvelines, France). Densitometry analysis of immunoblots involved use of Multi Gauge software (Fujifilm, Paris, France).

GAG and MMP enzymatic activity assays

Amount of GAG and global MMP activity were measured in the culture supernatants of compressed cartilage explants. GAG released was assessed by reaction with dimethylmethylene blue [33]. Shark chondroitin sulfate was used as a standard. MMP activity was assessed by Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 synthetic fluorogenic substrate (Bachem, Weil am Rhein, Germany) in continuous assays [34]. Results were normalized to milligram wet weight cartilage and concentration of proteins in the culture supernatant.

β-catenin accumulation

Intracellular proteins were extracted from chondrocytes cultured with and without IL-1β. Assay of total mouse β-catenin involved western blot analysis with rabbit polyclonal antibody for β-catenin (Cell Signaling, Danvers, Massachusetts). A re-probing with anti-β-actin antibodies (AC-15) (Sigma Aldrich) served as a loading control. Densitometry analysis of immunoblots involved use of Multi Gauge software (Fujifilm, Paris, France). The ratio of β-catenin band intensity to the β-actin band intensity was calculated.

Statistical analysis

Data are expressed as mean ± SEM and were analyzed by the use of Mann–Whitney non-parametric tests. 3 to 4 independent experiments were performed. P values (p) and number of experiments (n) are indicated in the figure legends.

Results

Load-induced catabolism is enhanced in FrzB$^{-/-}$ cartilage

Cartilage explants from FrzB$^{-/-}$ and wild-type mice were stimulated by dynamic compression for 6 h. As previously published by our group, load induced cartilage degradation in the explants [35]. Cartilage degradation was revealed by a net increase in GAG release (2.8-fold, $p = 0.014$, Figure 1A) and MMP activity (4.5-fold, $p = 0.014$, Figure 1B). At baseline, cartilage degradation was not different between the FrzB$^{-/-}$ and wild-type samples (data not shown). Load-induced GAG release was similar in FrzB$^{-/-}$ and wild-type samples but load-induced increase in MMP activity was slightly enhanced in FrzB$^{-/-}$ cartilage explants compared to wild-type (1.6-fold, $p = 0.17$, Figure 1B). Thus, the lack of FrzB tends to enhance the catabolic response of chondrocytes *in situ*, in response to mechanical stress.

Figure 1 Load-induced GAG release and MMP activity in mouse cartilage explants from FrzB$^{-/-}$ and wild-type (WT) mice. Explants were subjected to dynamic compression for 6 h (0.5 Hz, 1 MPa). FrzB$^{-/-}$ cartilage explants (black bars, n = 4) or WT ones (grey bars, n = 4) were loaded. Results from the loaded cartilage explants were normalized to the ones from the corresponding non-loaded explants (Ctrl), so that the graphs represent the fold-induction in response to compression. **A**, Amount of glycosaminoglycan (GAG) released from cartilage explants into culture supernatant. **B**, MMP enzymatic activity was measured in the culture supernatant of cartilage explants. Load-induced MMP activity tends to be enhanced in FrzB$^{-/-}$ explants compared to WT ($p = 0.17$). Bars represent the mean ± SEM, * $p \leq 0.05$ versus Ctrl.

IL-1β-mediated MMP induction is enhanced in FrzB$^{-/-}$ chondrocytes

Primary cultures of articular chondrocytes from FrzB$^{-/-}$ and wild-type mice were stimulated with increasing doses of IL-1β for 24 h (10, 100 and 1000 pg/mL). IL-1β dose-dependently induced *Mmp-13* gene expression (up to 15-fold, $p = 0.05$, Figure 2A). At the higher dose, IL-1β-induced *Mmp-13* gene expression was enhanced in FrzB$^{-/-}$ chondrocytes compared to wild-type ($p = 0.05$). Of note, in basal conditions, *Mmp-13* gene expression was similar in FrzB$^{-/-}$ and wild-type chondrocytes (0.012 ± 0.008 versus 0.020 ± 0.008 arbitrary units, respectively). At protein level, MMP-13 release was measured by western blot analysis (Figure 2B). MMP-13 release was not modulated by the lower doses of IL-1β, but was significantly increased in response to 1000 pg/mL ($p = 0.014$). This increase in MMP-13 release tended to be slightly enhanced in FrzB$^{-/-}$ chondrocytes compared to wild-type (1.5-fold, $p = 0.17$). Similarly, IL-1β dose-dependently induced *Mmp-3* gene expression (up to 500-fold, $p = 0.05$, Figure 3A). At the higher dose, IL-1β-induced *Mmp-3* gene expression tended to be enhanced in FrzB$^{-/-}$ chondrocytes compared to wild-type ($p = 0.10$). Alike for *Mmp-13*, *Mmp-3* gene expression was similar in FrzB$^{-/-}$ and wild-type chondrocytes in basal conditions (0.0021 ± 0.0010 versus 0.0017 ± 0.0004 arbitrary units, respectively). At protein level, MMP-3 release was assessed by ELISA (Figure 3B). MMP-3 was not detected in untreated conditions, but only in response to the higher doses of IL-1β ($p = 0.05$). MMP-3 release in response to 100 pg/mL of IL-1β was enhanced in FrzB$^{-/-}$ chondrocytes compared to wild-type (2.1 fold, $p = 0.10$). Thus, the absence of FrzB tends to enhance the catabolic response of cultured chondrocytes, in response to a pro-inflammatory stress. These results are parallel to the enhanced catabolic response observed in loaded FrzB$^{-/-}$ cartilage explants.

Figure 2 IL-1β-mediated increase in MMP-13 in cultured articular chondrocytes is enhanced in absence of FrzB. Primary chondrocytes were treated for 24 h with IL-1β (10, 100 or 1000 pg/mL). Results from the treated chondrocytes were normalized to the ones from non-treated samples (Ctrl), so that the graphs represent the fold-induction in response to IL-1β. **A**, Real-time PCR analysis of *Mmp-13* gene expression. IL-1β-induced *Mmp-13* gene expression tends to be enhanced in FrzB$^{-/-}$ chondrocytes compared to WT, especially with 1000 pg/mL ($p = 0.05$, $n = 3$). **B**, Culture media were analyzed for MMP-13 by Western-blotting. Quantification of MMP-13 blot is shown below the blot. IL-1β-induced MMP-13 protein release tends to be enhanced in FrzB$^{-/-}$ chondrocytes compared to WT, especially with 1000 pg/mL ($p = 0.17$, $n = 4$). Bars represent the mean ± SEM, * $p \leq 0.05$ versus Ctrl, # $p \leq 0.10$ between FrzB$^{-/-}$ and WT, ND = not detected.

Figure 3 IL-1β-mediated increase in MMP-3 in cultured articular chondrocytes is enhanced in absence of FrzB. Primary chondrocytes were treated for 24 h with IL-1β (10, 100 or 1000 pg/mL). **A**, Real-time PCR analysis of *Mmp-3* gene expression. Results from the treated chondrocytes were normalized to the ones from non-treated samples (Ctrl), so that the graphs represent the fold-induction in response to IL-1β. IL-1β-induced *Mmp-3* gene expression tends to be enhanced in $FrzB^{-/-}$ chondrocytes compared to WT, especially with 1000 pg/mL ($p = 0.10$, $n = 3$). **B**, Culture media were analyzed for total MMP-3 by ELISA. MMP-3 protein release in response to 100 pg/mL of IL-1β tends to be enhanced in $FrzB^{-/-}$ chondrocytes compared to WT ($p = 0.10$, $n = 3$). Bars represent the mean ± SEM. * $p \leq 0.05$ versus Ctrl, # $p \leq 0.10$ between $FrzB^{-/-}$ and WT, ND = not detected.

IL-1β- and load-induced catabolism in $FrzB^{-/-}$ chondrocytes is associated with canonical Wnt/β-catenin signaling

We wondered if the enhancement in the response of $FrzB^{-/-}$ chondrocytes to IL-1β and load was associated with a deregulation in Wnt/β-catenin signaling. In basal conditions, *Ctnnb1* gene expression (coding β-catenin) was comparable between $FrzB^{-/-}$ cultured chondrocytes and wild-type ones (0.62 ± 0.22 versus 0.95 ± 0.12 arbitrary units, respectively). Of note, similar results were observed for the Wnt/β-catenin target gene *Lef1* (0.031 ± 0.009 for wild-type versus 0.018 ± 0.013 for $FrzB^{-/-}$ chondrocytes). These data are in accordance with the previous transcriptome analysis of the Bone-Cartilage unit of $FrzB^{-/-}$ mice : at baseline *in vivo*, *Ctnnb1* mRNA level was not different between the $FrzB^{-/-}$ and wild-type strain [36].

Ctnnb1 gene expression was not affected by IL-1β in wild-type cultured chondrocytes (Figure 4A). In IL-1β-treated $FrzB^{-/-}$ chondrocytes, *Ctnnb1* gene expression was induced 4.3-fold compared to untreated ones ($p = 0.014$). Thus, *Ctnnb1* gene expression was markedly higher in IL-1β-treated $FrzB^{-/-}$ chondrocytes compared to wild-type ones (3.6 fold, $p = 0.057$). In parallel, *Lef1* gene expression was induced 6.2-fold in IL-1β-treated $FrzB^{-/-}$ chondrocytes compared to untreated ones ($p = 0.05$) and *Lef1* mRNA level was clearly higher in IL-1β-treated $FrzB^{-/-}$ chondrocytes compared to wild-type ones (13 fold, $p = 0.05$, Figure 4A). Analysis of β-catenin accumulation at protein level did not strictly correlate this transcriptional regulation (Figure 4B). IL-1β tended to decrease β-catenin content of wild-type cultured chondrocytes (20% less, $p = 0.057$), whereas no modulation was observed in $FrzB^{-/-}$ ones. Thus, β-catenin content was higher in IL-1β-treated $FrzB^{-/-}$ chondrocytes compared to wild-type ones (1.4 fold, $p = 0.10$). IL-1β only slightly modulates Wnt/β-catenin signaling in our model, but a FrzB-dependent deregulation was observed in IL-1β-treated chondrocytes.

Figure 4 IL-1β- and load-mediated regulation of Wnt/β-catenin signaling in chondrocytes from $FrzB^{-/-}$ and wild-type (WT) mice. A, B, Cultured articular chondrocytes from $FrzB^{-/-}$ mice or WT ones were treated for 24 h with IL-1β (1 ng/mL). Results from the IL-1β-treated samples were normalized to the control ones (Ctrl), so that the graphs represent the fold-induction in response to IL-1β. **A**, *Ctnnb1* gene expression (coding β-catenin) and *Lef1* gene expression (a Wnt/β-catenin target gene) were analyzed by real-time PCR ($n = 4$ and $n = 3$, respectively). *Ctnnb1* gene expression was not modulated by IL-1β in WT chondrocytes and *Lef1* gene expression was decreased ($p = 0.05$). In contrast, the treatment induced *Ctnnb1* and *Lef1* gene expression in $FrzB^{-/-}$ chondrocytes ($p = 0.014$ and $p = 0.05$, respectively). Thus IL-1β-mediated regulation of β-catenin expression was different between $FrzB^{-/-}$ and WT ($p = 0.057$ for *Ctnnb1*, $p = 0.05$ for *Lef1*). **B**, Intracellular extracts were analyzed for total β-catenin by Western-blotting. Quantification of β-catenin blot

suggested that IL-1β-mediated β-catenin accumulation was different between $FrzB^{-/-}$ and WT ($p = 0.10$, $n = 4$). **C**, $FrzB^{-/-}$ cartilage explants or WT ones were subjected to dynamic compression for 6 h (0.5 Hz, 1 MPa). *Ctnnb1* gene expression was analyzed by real-time PCR ($n = 3$). Results from the loaded cartilage explants were normalized to the ones from the corresponding non-loaded explants (Ctrl), so that the graphs represent the fold-induction in response to compression. *Ctnnb1* gene expression was not affected by load in WT explants but was increased 2.7-fold in compressed $FrzB^{-/-}$ samples ($p = 0.05$). Load-induced increase in *Ctnnb1* mRNA tends to be enhanced in $FrzB^{-/-}$ explants compared to WT ($p = 0.20$). Bars represent the mean ± SEM, * $p \leq 0.05$ versus Ctrl, # $p \leq 0.10$ between $FrzB^{-/-}$ and WT.

Concerning the catabolic response of $FrzB^{-/-}$ cartilage to load, analysis of *Ctnnb1* gene expression showed very similar results (Figure 4C). *Ctnnb1* mRNA level was not affected by compression in wild-type explants. In compressed $FrzB^{-/-}$ samples, *Ctnnb1* gene expression was induced 2.7-fold compared to uncompressed ones ($p = 0.05$). Thus, the modulation in Wnt/β-catenin signaling in response to load was slightly enhanced in $FrzB^{-/-}$ cartilage ($p = 0.20$, Figure 4C).

Overall, these results suggest that the enhancement in the response of $FrzB^{-/-}$ chondrocytes to IL-1β and load may be associated with an over-stimulation of the canonical Wnt pathway.

Discussion

Enhanced responsiveness to mechanical stress in absence of FrzB and involvement of canonical Wnt/β-catenin pathway

We demonstrated here that load-induced MMP activity was enhanced in $FrzB^{-/-}$ cartilage explants. In addition, load stimulated *Ctnnb1* gene expression in $FrzB^{-/-}$ explants. These results suggest that the load-induced catabolic response of chondrocytes may be in part mediated by the canonical Wnt pathway. The involvement of Wnt signaling in osteoblast responsiveness to mechanical stimulation was proven in various studies [37-42]. In particular, TOPGAL reporter mice showed that the canonical Wnt/β-catenin pathway was activated by mechanical stress *in vivo* and in cultured osteoblasts [43]. Furthermore, increasing basal β-catenin levels was shown to enhance the effects of mechanical stress [44]. Chondrocyte responses towards mechanical stimulation have been less studied. However, pressure-induced mechanical stress triggered β-catenin tyrosine phosphorylation in cultured chondrocytes [45] thereby likely releasing the molecule from adherens junctions and increasing its availability for intracellular signaling. Partial β-catenin nuclear translocation was also observed in response to tensile strain [27]. Furthermore, there was an additive effect of load and Wnt3a on β-catenin translocation and on up-regulation of *Mmp-3* gene expression [27]. Of interest, a down-regulation of FrzB was observed in human and mouse cartilage explants in response to mechanical injury, suggesting a de-repression of canonical Wnt pathway [46]. Thus, Wnt/β-catenin signaling may be part of the signaling response leading to excessive catabolism and cartilage degradation in response to abnormal loading and FrzB may have a protective role on load-induced increase in MMP activity.

Enhanced responsiveness to IL-1β in absence of FrzB and involvement of canonical Wnt/β-catenin pathway

We demonstrated that IL-1β-mediated increases in *Mmp-3* and *−13* gene expression and protein release were enhanced in $FrzB^{-/-}$ chondrocytes. Similarly, IL-1β stimulated *Ctnnb1* and *Lef1* gene expression and β-catenin accumulation in $FrzB^{-/-}$ chondrocytes. Canonical Wnt pathway activation may thus enhance chondrocyte responsiveness to IL-1β. These results suggest a crosstalk between the canonical Wnt pathway, which includes β-catenin and FrzB, and IL-1 pathway, which stimulates MMP expression in chondrocytes. In accordance with our results, activation of β-catenin signaling in cultured chondrocytes by Wnt3a treatment potentiated IL-1β-mediated loss of proteoglycans [25]. Conversely, inhibition of β-catenin signaling by the use of Lef1 siRNA down-regulated IL-1β-mediated increase in *Mmp-13* gene expression [29]. Also, Sost, which is a biologically active inhibitor of β-catenin signaling in chondrocytes, down-regulated IL-1α-mediated increase in *Mmp-13* gene expression in cartilage explants and also reduced the loss of proteoglycans [47]. An alternative suggestion is that IL-1β treatment may induce canonical Wnt production, such as Wnt-7b, which in turn could activate β-catenin signaling [48]. In surprising contrast, in human chondrocytes, β-catenin signaling was found to counteract IL-1β-mediated increase in MMP-3 and −13 expressions [48]. In conclusion, canonical Wnt pathway may be part of mechanisms leading to excessive catabolism in response to inflammatory stress and FrzB may have a protective role on IL-1-mediated increase in MMP expression in mouse chondrocytes.

Putative protective role of FrzB in OA progression

In osteoarthritis (OA), cartilage breakdown is due to cleavages of matrix molecules in response to abnormal mechanical stress and to some degree of inflammation. Because our results suggest that FrzB may have a protective role on load- and IL-1-mediated catabolic processes in mouse chondrocytes, we speculate that FrzB may have a protective role in OA. Our results are consistent with the increased cartilage loss observed in models of arthritis in $FrzB^{-/-}$ knockout mice [18]. Moreover, gene expression of *Mmp-3* was up-regulated in the cartilage of $FrzB^{-/-}$ mice with mBSA-induced arthritis compared with wild-type mice. However, $FrzB^{-/-}$ knockout mice did not develop spontaneous OA, and they did not show aberrant *Mmp* gene expressions in basal conditions, except a 2-fold increase for MMP-3 [36]. Thus, FrzB may be involved in OA progression rather than OA onset. Although FrzB may interact directly with MMP-3 [18], its protective role is probably linked with a deregulation of the Wnt pathways. The recent identification of FrzB as a blocker of hypertrophic differentiation in articular cartilage [22], promote the hypothesis of a protective role of FrzB in OA through the Wnt-mediated regulation of hypertrophic maturation.

Conclusions

Our results suggest that FrzB has a protective role on MMP induction in mouse chondrocytes. They indicate a dual role of Wnt signaling in cartilage homeostasis, so that a controlled amount of Wnt signaling is necessary for maintenance of the articular cartilage, but an excess one is deleterious. Further investigations are needed to decipher the tight control of Wnt signaling in OA, in particular concerning the differentiation of OA chondrocytes towards hypertrophy.

Our results also add evidence to demonstrate that the canonical Wnt/β-catenin pathway is part of mechanisms leading to excessive catabolism and cartilage degradation in OA. However, the FrzB-dependent deregulation that we observed may involve both canonical and non-canonical Wnt signaling since FrzB is an inhibitor of both pathways. Little is known concerning the role of non-canonical Wnt pathway in articular cartilage homeostasis and OA development. Recent data suggested that, in excess, Wnt5a could stimulate degradation of mature cartilage matrix *via* non-canonical pathways, while promoting normal differentiation in developing cartilage [49]. Therefore, additional investigations on Wnt regulation in OA should equally explore canonical and non-canonical Wnt pathways.

Abbreviations

ELISA: Enzyme-linked immunosorbent assay; FrzB: Frizzled-related protein B: also called secreted Frizzled-related protein 3; GAG: Glycosaminoglycan; IL: Interleukin; KO: Knockout; MMP: Matrix metalloproteinase; OA: Osteoarthritis; WT: Wild-type

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Conception and design: CB, SP, XH, CJ, FB. Acquisition of data: CB, SP, AP, LS. Analysis and interpretation of the data: CB, SP, XH, RL, CJ, FB. Drafting of the article: CB, SP, AP. Critical revision of the article for important intellectual content: XH, RL, CJ, FB. Final approval of the article: CB, SP, XH, AP, LS, RL, CJ, FB.

Authors' information

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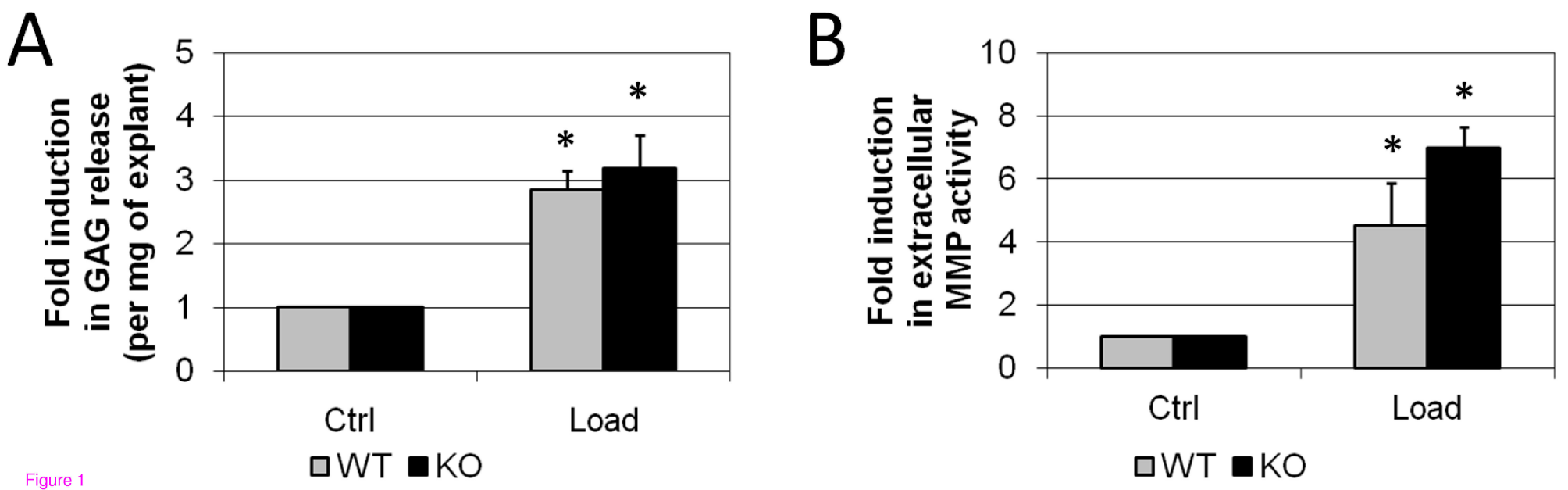
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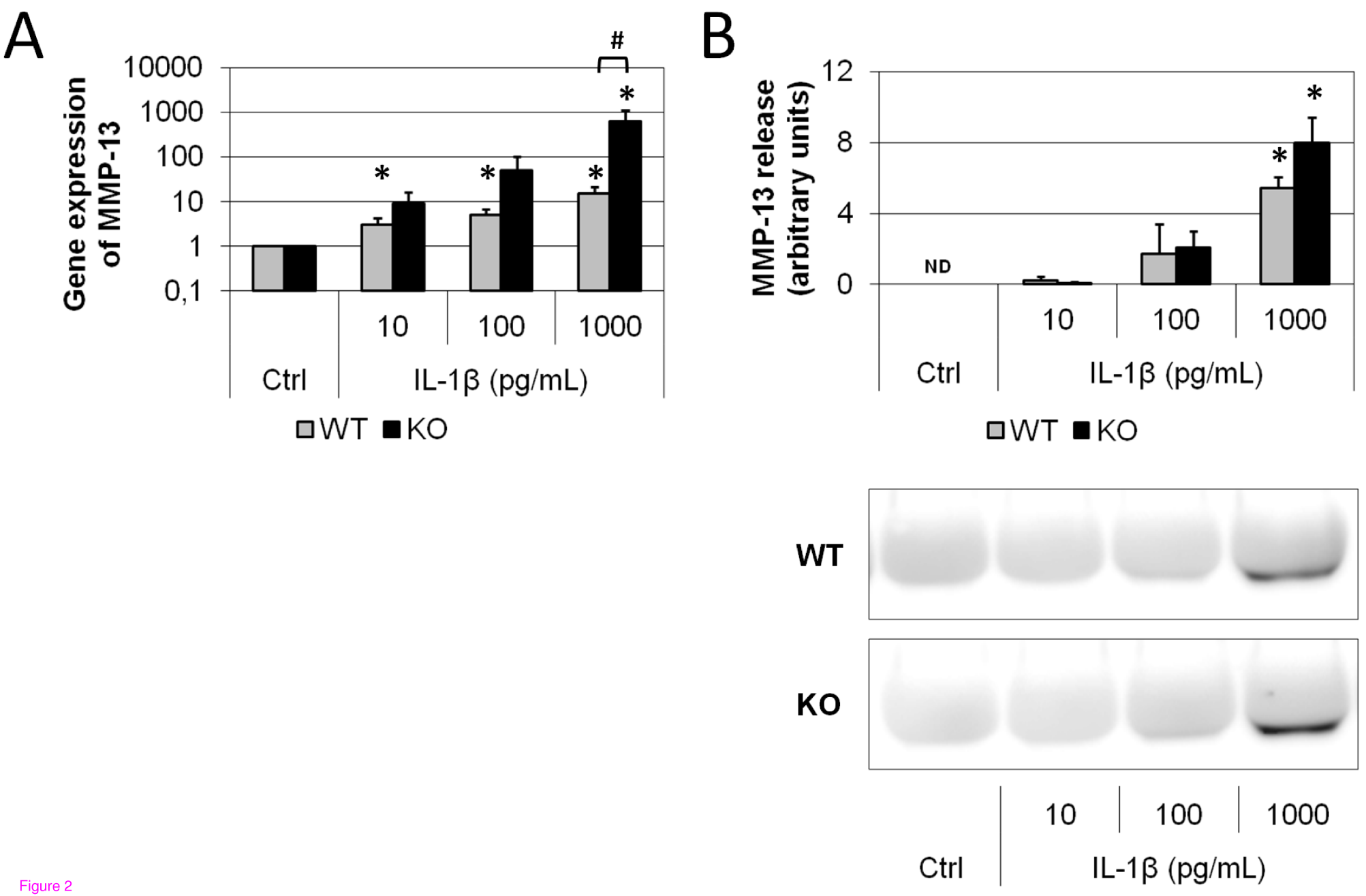
Figure 1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IL-1β (pg/mL)
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MMP-13 release (arbitrary units)
12
8
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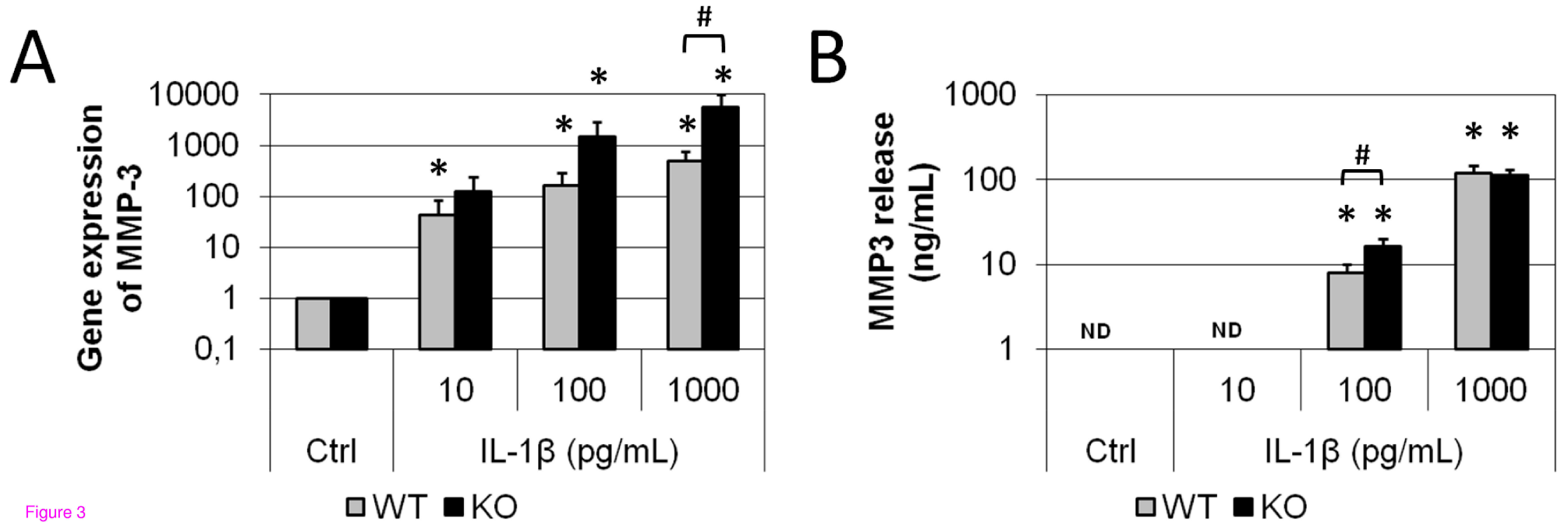
Figure 2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IL-1β (pg/mL)
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MMP3 release (ng/mL)
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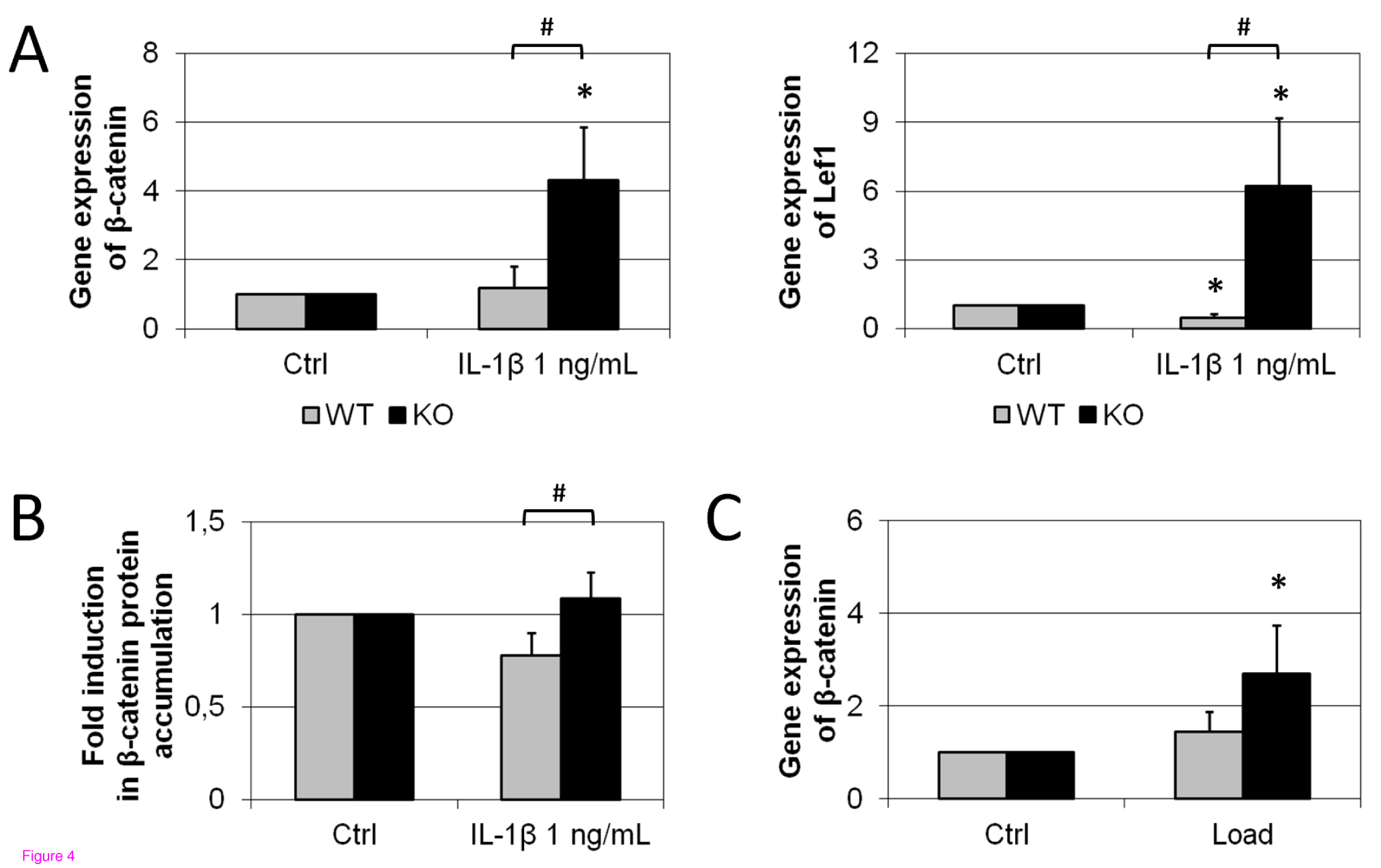
Figure 3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Gene expression of β-catenin
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KO
Gene expression of Lef1
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9
6
3
0
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*
Ctrl
IL-1β 1 ng/mL
WT
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B
Fold induction in β-catenin protein accumulation
1,5
1
0,5
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Ctrl
IL-1β 1 ng/mL
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Gene expression of β-catenin
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4
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Figure 4